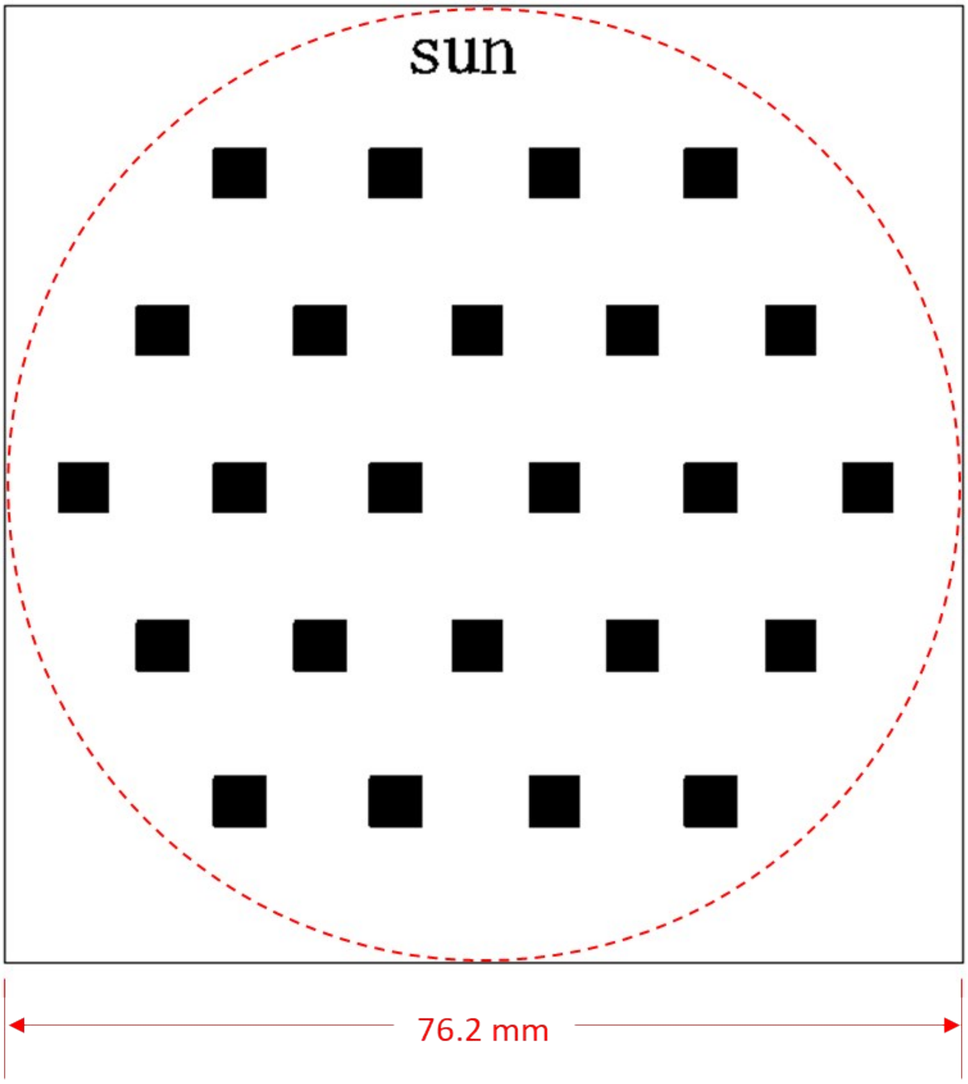
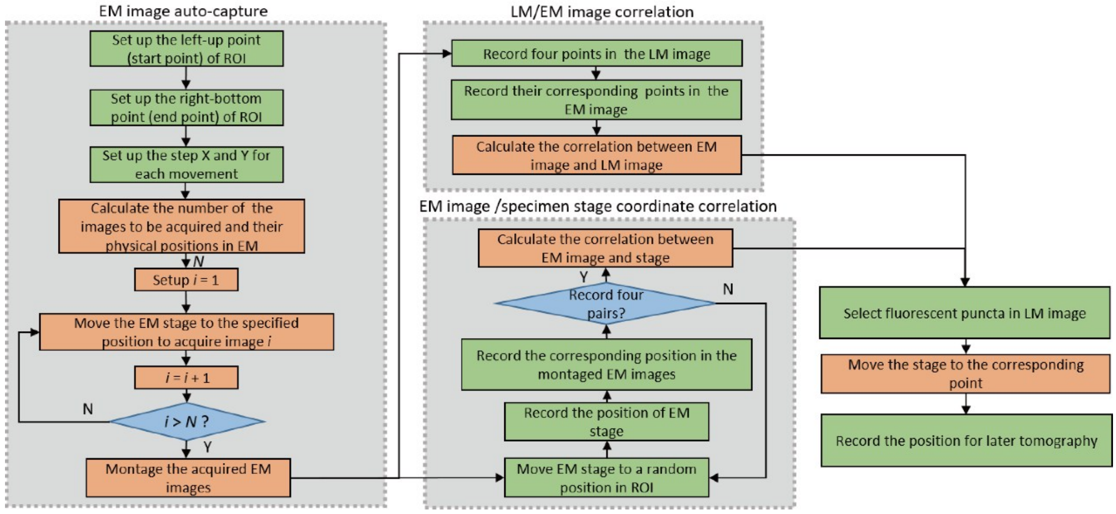
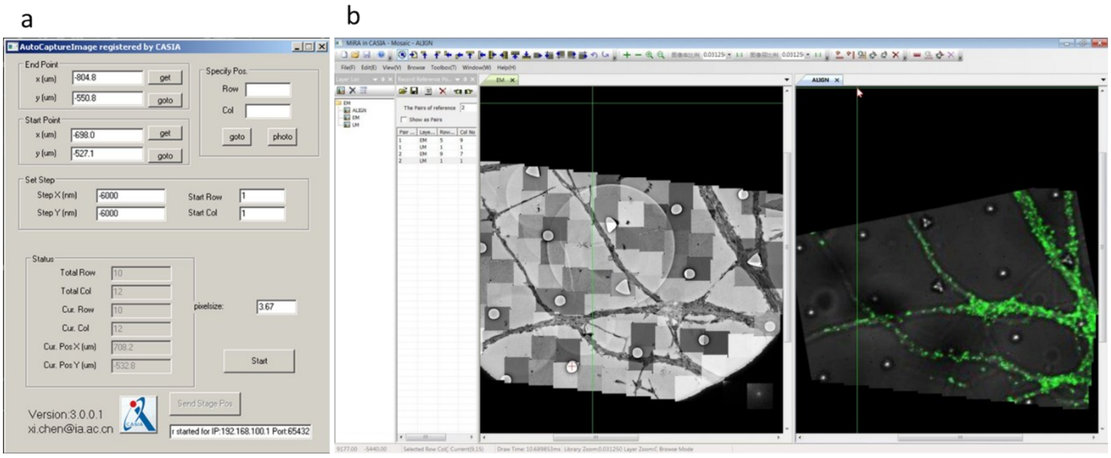
Electronic Supplementary Material



Supplementary Fig. 1. Drawing of the entire photomask. The square mask consists of 24 repeated elements. The individual element is shown in Fig. 2a for one PDMS substrate. The photoresist mold is fabricated on the round silicon wafer with a diameter of 76.2 mm (3 in).



Supplementary Fig. 2. Block diagram of the custom-developed CLEM software package.



End Point
x (um) -804.8
y (um) -550.8

Start Point
x (um) -698.0
y (um) -527.1

Set Step
Step X (nm) -6000
Step Y (nm) -6000
Start Row 1
Start Col 1

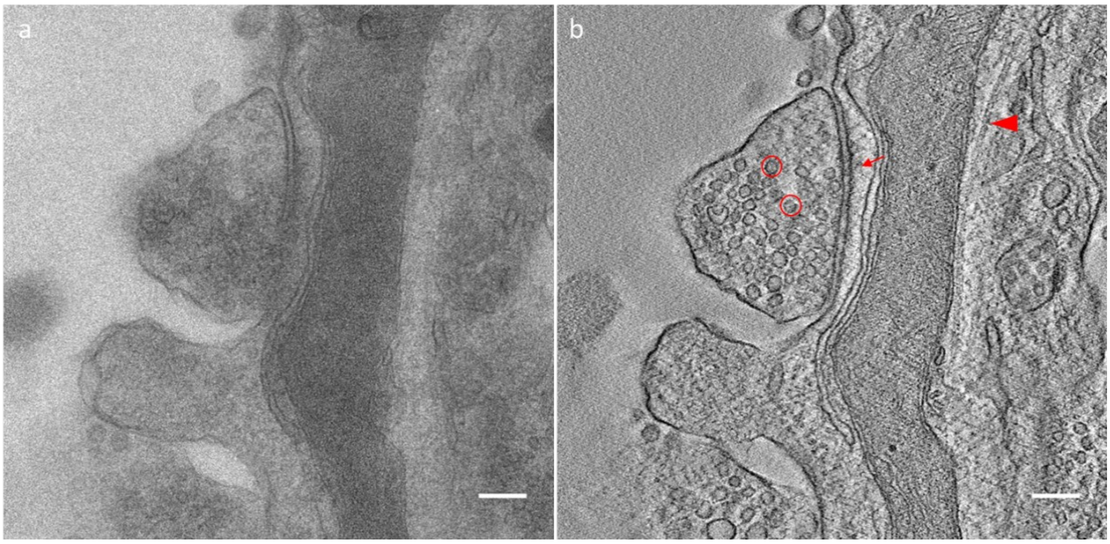
Status
Total Row 10
Total Col 12
Cur. Row 10
Cur. Col 12
Cur. Pos X (um) 708.2
Cur. Pos Y (um) -532.8

pixelsize: 3.67

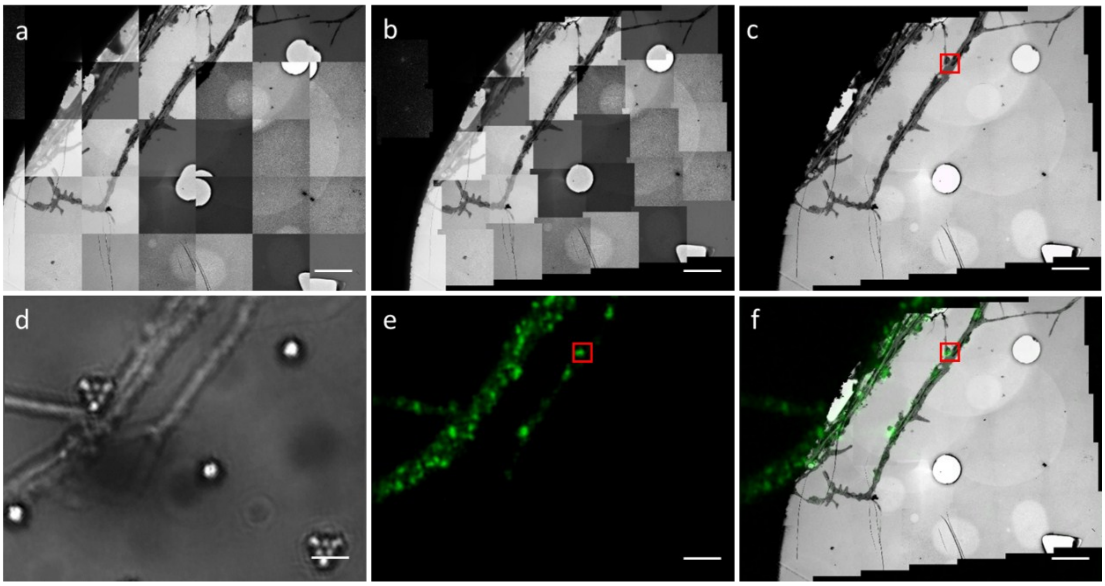
Version:3.0.0.1
xi.chen@ia.ac.cn

r started for IP:192.168.100.1 Port:65432

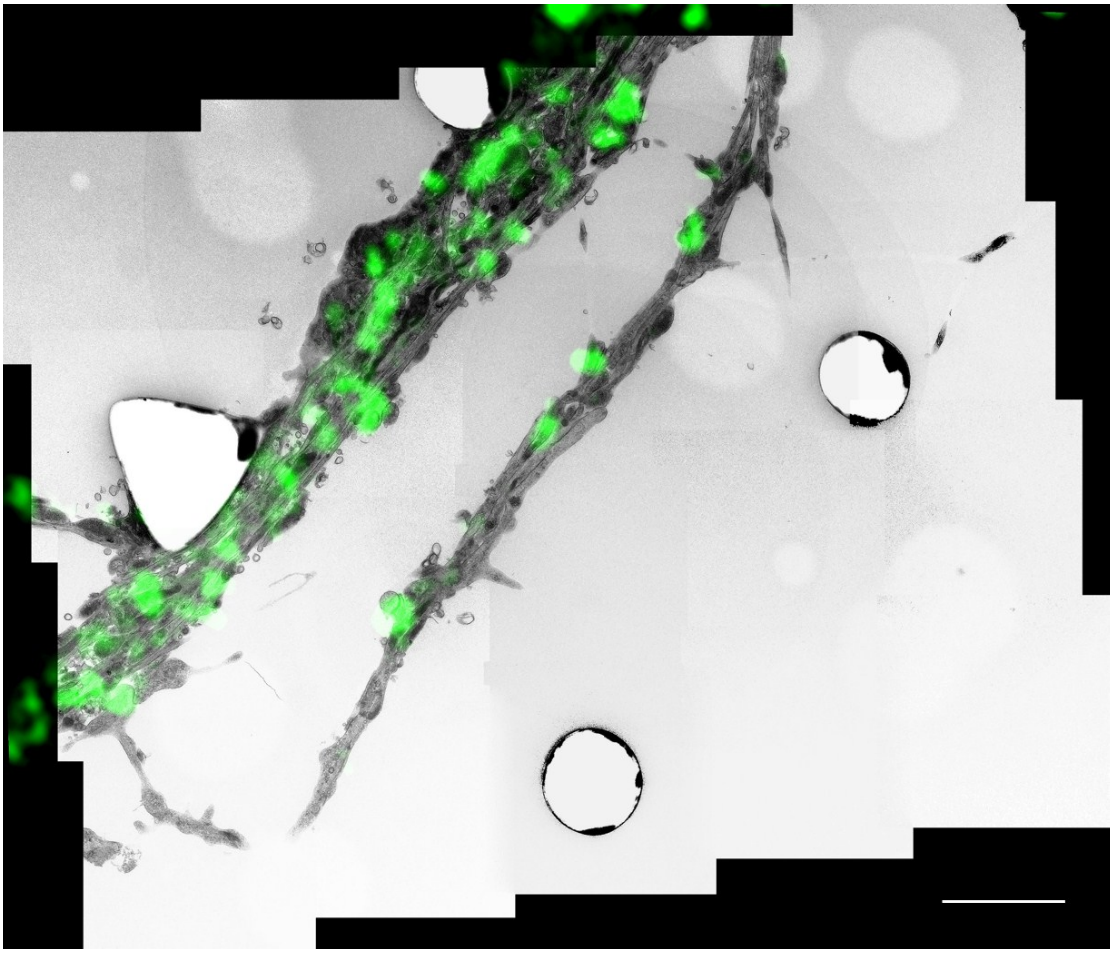
Supplementary Fig. 3. CLEM software graphical user interface. (a)The image capture program. (b) The mosaic program.



Supplementary Fig. 4. A single projection image and a tomographic slice showing the same shaft synapse. Circles, synaptic vesicles; arrow, PSD; arrow head, microtubule. Scale bars, 100 nm.



Supplementary Fig. 5. CLEM procedure of the synapse in Fig. 4h3 and 4h4. (a-c) Raw (a), montaged (b), and contrast-adjusted (c) image of sequential EM images. (d and e) Bright field (d) and fluorescent (e) image of the same view field with EM image. (f) EM and LM images are correlated and merged by aligning designed PDMS patterns. Boxed areas indicate the synapse shown in Fig. 4h3 and 4h4. Scale bars, 5 μm.



Supplementary Fig. 6. A high resolution version of Fig. 4f. Scale bar, 5 µm.